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MINI REVIEW

A short review of the pinewood nematode, *Bursaphelenchus xylophilus*

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Abstract

Objective and methods This article provides a summary of studies on pine wilt disease (PWD). PWD is a serious threat to forests, and the damage caused by this disease results in significant economic loss. In addition, PWD adversely affects not only animals and plants, but also the human environment. Having a better understanding over all possible interference and control measures strategies derived from knowledge of the complicated interrelation between the nematode, its vectors and the host pine trees is a precondition to effectively reduce the damage caused by the pine wood nematode (PWN). The references in this paper were collected from various sources, including PubMed, Google Scholar, and Web of knowledge before being organized by the authors.

Results and conclusion Most papers discussing PWD have been conducted on the East Asia and European Union regions. Specific topics covered include: (1) damage and invasion of pine wilt disease, (2) the developmental cycle and transmission, (3) diagnosis method for PWN related to PWD and (4) control strategies to limit the spread of PWD.

Keywords Pinewood nematode · Diagnosis · Control strategies

Introduction

The pine wood nematode (PWN), *Bursaphelenchus xylophilus*, is a lethal pest that infects pine wood trees. Although PWN supposedly originated in North America, pine wilt disease (PWD) was first recorded in Japan in the early twentieth century [1, 2]. Subsequently, the disease has spread to other East Asian countries including Korea and China. Asian pinus species, including *P. massoniana*, *P. densiflora* and *P. thunbergii*, are susceptible to *B. xylophilus* as a high-risk species. As a result, PWD, which is caused by *B. xylophilus*, has caused extensive damage in pine forests of East Asian countries, in particular Japan and Korea [3, 4]. In the 1990s, PWN was introduced into Portugal from East Asia where it caused major forest damage, and recently, it was introduced into Spain [5]. Accordingly, PWN is legally listed as a quarantine pest in many countries, and protecting pines against PWN is recognized as an urgent problem for forestry [6].

PWN is transmitted to dead or dying trees during activity of oviposition or maturation feeding of vector beetles. For example, *Monochamus* is a beetle species of a genus that is a major vector for *Bursaphelenchus* sp. [7, 8]. Significant efforts have been devoted to researching PWD, including investigations of the phoretic relationship between PWN and its insect vector as well as methods for diagnosis on PWN. These include review papers that provide an overview of PWD, how host pine trees respond to PWN infection, the infection history of PWD, and how to prevent the spread of PWD. Globalization and climate change are also increasing the opportunity for further incursion and expansion of PWN around the world. As such, we now recognize the importance of PWD, and research on PWD should continue.

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Damage and invasion by pine wilt disease

An understanding of pine wilt disease (PWD) necessitates understanding the history of the invasion process. PWD is caused by the pine wood nematode (PWN), *Bursaphelenchus xylophilus*, and it results in an annual loss of millions of pine trees [9, 10]. PWN is thought to have originated in North America, namely Canada and the USA, and the first occurrence of PWD that resulted in damage to the pine forests of Japan was reported in 1905 [11]. Also, PWD caused by PWN, *B. xylophilus*, was first identified in Japan. Since then, the disease has spread from Japan to neighboring East Asian countries of China, Taiwan and Korea in 1980s [12–15]. In Europe, it was first observed in Portugal in 1999, it was also detected in Nigeria and Mexico in 1990s, [16, 17] and it has since been found to cause PWD in Spain from 2008 (Fig. 1) [18, 19].

PWN, *B. xylophilus*, causes extensive damage to the pine forests of East Asian countries, specifically, Japan and Korea, because it occupies many pine species vulnerable to PWD. Since the introduction of PWD, the disease has led to a high mortality in pine trees in Japan for the past hundred years, and the annual loss of pine coverage has been more than 50 million m^3 in 10 years from 2004 to 2014 (Fig. 2). To date, the total financial loss due to PWD in Japan has been estimated at 3.7 billion US dollars, assuming a market price of pine trees of US \$100/m^3 [20]. Pine trees infected with PWD were first discovered in Busan, Korea, in 1988. Due to strict control efforts during the period of severe invasion, the areas with major damage were limited to the southern regions in the twentieth century [21]. However, PWD has begun to gradually spread to the northern area of the country since 2010 as the average temperature has increased (Fig. 3). As a result, the number of pines infected by PWD has increased sharply since 2011, resulting in the largest losses of forest damage of 1.74 million pine trees in 2015. Since then, the number of pines infected with PWD decreased to less than half in 2019 due to intensive management. According to the Korean Forest Service, the total damage to forest products caused by PWD in the last decade is estimated to reach 8.4 billion won. In addition, environmental damages including loss of biodiversity and the cost of PWD control systems represent an even larger economic loss due to pine disease [22]. Moreover, even with quarantine efforts such as the restricted import of wood products to prevent PWN from reaching European countries, the disease has been found its way to Portugal and northern Spain. It has been predicted that by 2030, PWD could spread over 8–34% of Europe, and if PWN is not controlled, the cumulative value of lost forestry stock is estimated to reach € 22 billion [23, 24]. Therefore, PWD has become one of the most serious forest diseases in the world [25, 26].

The developmental cycle and transmission

Enormous efforts have been dedicated to studying PWD, including the pathological events after PWN infections and the phoretic relationship between the PWN and its vector

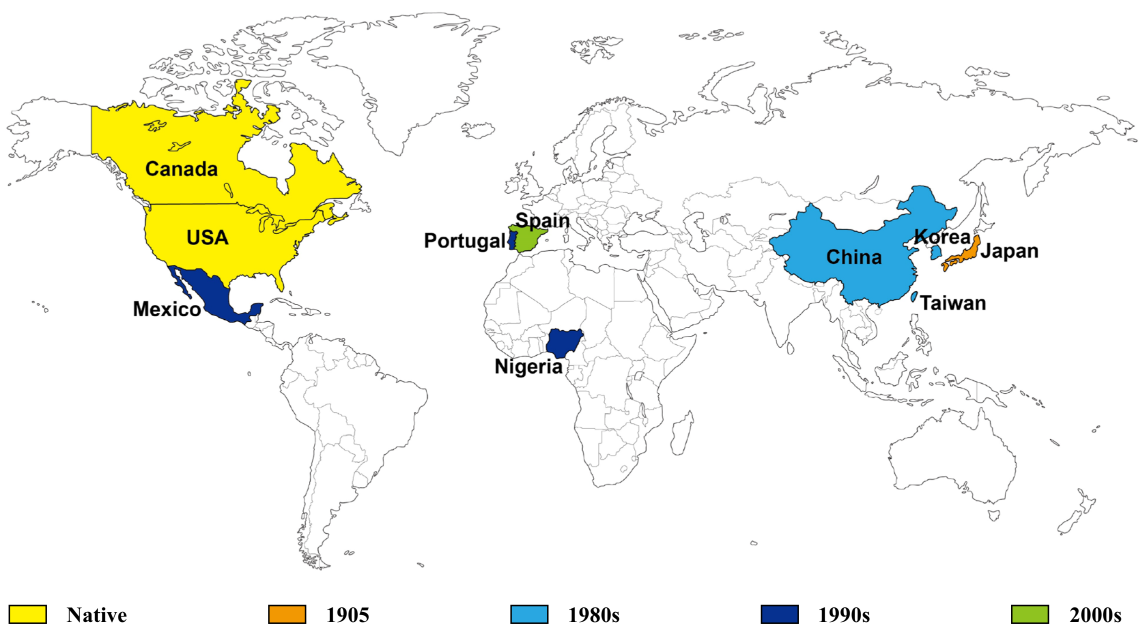

Fig. 1 History of the invasion of pine wilt disease around the world. Pine wood nematode, *Bursaphelenchus xylophilus*, is probably native to America. *B. xylophilus* was first reported in Japan and has spread to other countries

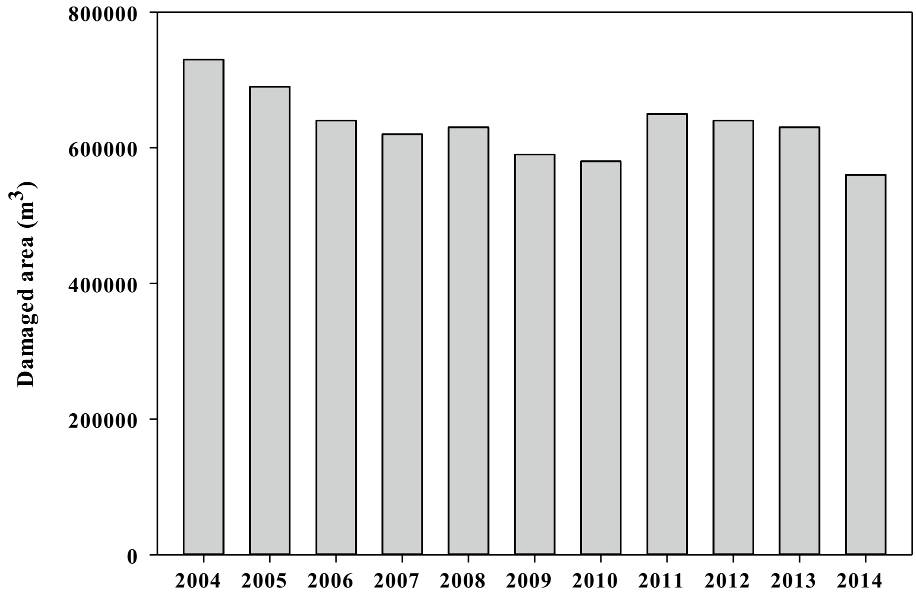

Fig. 2 Sequential changes in areas damaged by pine wilt disease from 2004 to 2014 in Japan. A PWD-damaged area was present each year. These data were provided by the Forestry Agency (2015) in Japan

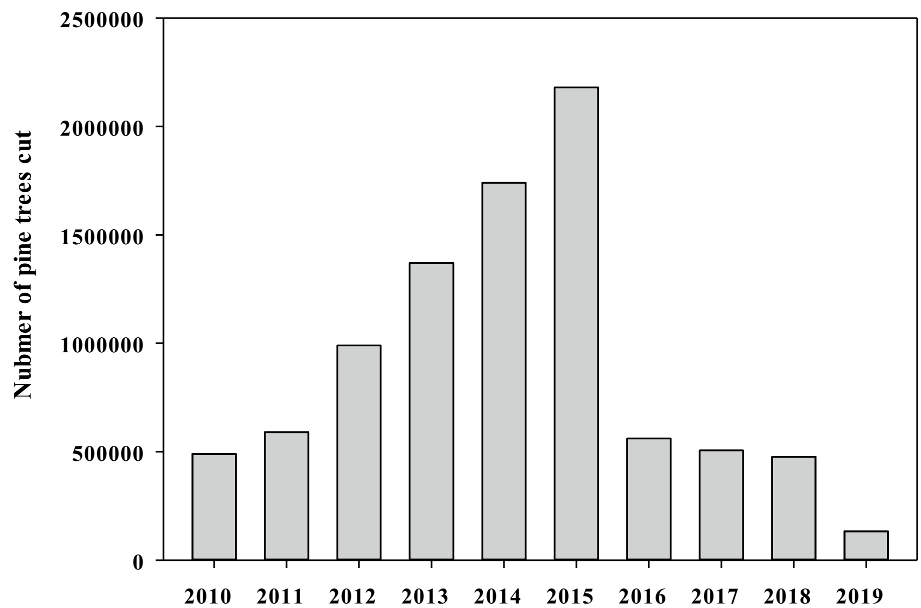

Fig. 3 Annual changes in the number of pine trees felled by pine wilt disease in Korea. PWD-infected trees were present in each year. These data were provided by the Korea Forest Service

beetles. These comprehensive research efforts have been able to determine the developmental cycle of PWD. The nematode developmental cycles shown in Fig. 4 illustrate how nematodes develop through four juvenile stages and reproduce within wood tissue, while food is available (which is called the propagative cycle). The first juvenile stage (J_1) molts to the second juvenile stage (J_2) in the egg. J_2 hatches from the egg, and there are two more juvenile stages (J_3 and J_4) preceding the adult. Different types of juvenile stages appear under different conditions. In this life cycle, when the conditions are unfavorable and reach the dispersal cycle, PWN appears with the specialized third-stage juvenile (J_{III}). When the environment becomes dry or adverse because of nematode overpopulation, J_{III} survive at a higher ratio than other propagative forms because of their unique body structure. [27] Generally, this stage molts into the fourth-stage juvenile (J_{IV}), which is transmitted by vector beetles to new trees [28–31]. However, in the absence of a suitable vector, the PWN population in the tree will ultimately die.

The development of PWN is greatly influenced by temperature and seasonality [32]. From May through June, the J_{IV} juveniles of the nematode invade the beetle's body. From early-June to late-July, the PWN infection of healthy pine trees occurs with adult beetles that have left dead pine trees [33, 34]. Under natural conditions, from mid-July, the population of propagative J_3-stage juveniles rapidly increases and

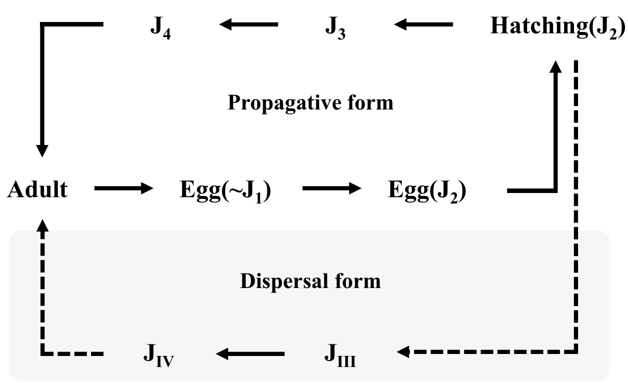

Fig. 4 The developmental cycles of *Bursaphelenchus xylophilus* under two conditions. The solid arrows and dashed arrow appear the propagation cycle in pine trees and that for transmission to new host trees by insect vectors, respectively. The life cycle of PWN has juvenile stages of J_1, J_2, J_3 and J_4 under favorable conditions, and juvenile stages of J_{III} and J_{VI} under unfavorable conditions [27]

reaches a maximum level as the pine wilt disease became more advanced. In November and later, the PWN population in the dead tree declines, but the proportion of J_{III} juveniles in the population increases. During the winter and into the spring, the dispersal J_{III}-stage juveniles gather around the pupal chambers of the beetles. Thus, the transmission of PWD is associated with the pine host and insect vectors [35–38].

Since the nematode cannot spread over a long distance by itself, it needs an insect vector [39–41]. The J_{III} aggregate around the pupal chambers of the vector beetle. They molt to the J_{IV} stage and then invade the insect vector's body. A vector of the PWN, *Monochamus* beetles including *Monochamus alternatus* in Asia, *M. carolinensis* in the North America, and *M. galloprovincialis* in Southern Europe move from dead to healthy pines for prolonged feeding on young branches until their reproductive organs mature [42–46]. Then, J_{IV} leave the beetle's body and invade new healthy trees. The *Monochamus* beetle is the most efficient vector for long distance transport of PWN. This results in PWN being transmitted to a new host tree or newly cut log by the beetle during oviposition, and then repeating the propagative cycle again.

Diagnosis method for PWN related to PWD

The economic loss in forests resulting from the invasion of PWN into new areas has highlighted a need for accurate diagnosis of this species to prevent further spread. Traditionally, the detection of PWN has been based on the morphological characteristics after their extraction from wood samples. The PWN has been identified according to three characteristics: (1) in the male, flattened spicules with a disc-like cucullus at the tip, (2) in the female, an anterior vulval lip with a distinct flap-like overlap, and (3) a tail or posterior end of the body of the females that is usually round [47]. However, this method of identification is time consuming and requires a high level of taxonomical expertise. Besides, the morphological identification of PWN can sometimes be difficult or impossible as the species of the genus *Bursaphelenchus* are similar in morphology [48]. Accordingly, the need for more sensitive and accurate methodologies has led to the development of molecular detection using several DNA-based protocols. Identification methods based on molecular biology require expensive equipment and reagents, but can be made to be simpler, faster and more reliable. Several PCR-based methods have been designed to detect PWN with species-specific probes by targeting either internal transcribed sequence (ITS), intergenic spacer (IGS) regions in the ribosomal DNA (rDNA), satellite DNA (satDNA), or restriction enzymes species-specific pattern (RFLP), and real-time PCR technology based on a heat shock protein gene (hsp70) [49–53]. More recently, morphologically similar *Bursaphelenchus* sp. could be detected using multiples RT-PCR capable of detecting multiple species simultaneously [54, 55]. Also, method for direct detection of PWN have been developed using loop-mediated isothermal amplification (LAMP) tests and PCR amplification of the species-specific Mspl satDNA [56, 57]. Therefore, to prevent the spread of PWN between countries and economic and biological forest loss, accurate techniques to detect and identify PWN are required by using appropriate morphological and molecular methods.

Control strategies for limitation of spreading of PWD

PWD causes significant ecological and economic losses in natural coniferous forests in Asia (especially in Japan, China, and Korea) and Southern Europe (especially Portugal). Accordingly, PWN is among the most important pests included in the quarantine lists of many countries around the world [58]. Due to this serious damage, many scientists have tried numerous methods to manage PWD. In order to protect pine trees from PWD, there are methods to (1) control the pine nematodes themselves, (2) control the insect vectors, and (3) increase the resistance to PWN. There are several ways to carry out these strategies [59, 60].

Physical control is a highly effective control method. Physical tactics such as felling, crushing and burning infected pine trees could be used for large-scale treatment. An advantage of crushing is that the sawdust and chips from the diseased trees can be used, but the product is expensive because it requires intensive labor and machines must move through the forests. Burning infected trees is the most efficient method to control PWN, but its use is restricted to periods when the forest fire risk is low. Also, heat from the fires can damage other pine trees [61]. Although treating infected trees or dead trees regarding the insect vector and PWN can be used to prevent the spread of PWD, it is not a method to control or treat PWD before PWN infection. Therefore, to prevent PWD infection, chemical control methods such as tree injections and spraying nematicides and insecticides are more realistic [62].

Chemical controls are also major strategies for eradication, and these have been used for prevention. To protect the pine tree from the pine wilt, there are methods to control the pine wood nematode and the insect vector. Recent researches have shown that bacteria associated with pinewood nematode (PWN) affect pine wilt. Phytotoxins secreted by bacteria associated with PWN might be involved in the pathogenesis of PWD by inducing damage plant cells [63]. To control the nematode, there are methods such as trunk injection of nematicides or spraying the ground, and the target of the insect vector can be controlled by spraying or fumigating insecticides. Some trunk-injection agents containing mesulfenfos, morantel tartrate, levamisole hydrochloride, abamectin or emamectin benzoate as the antinematodal ingredient can be applied to the pine tree trunk [64–68]. These agents are known to be directly effective against PWN and safe for the environment. However, avermectin has been reported to produce resistance in nematodes, although PWN has not yet been reported to show resistance [69, 70]. Controlling insect vectors could be a more efficient way to prevent the spread of PWD. This practical method can be applied to hard-to-reach places and can be applied to a wider area. To control the dispersal of vector beetles, one or several of prothiophos, fenitrothion, fenthion, pyridaphenthion, thiacloprid, or chlorpyrifos-methyl chemicals can be included as major components of the insecticides used for preventative spraying against vector beetles [71–73]. However, chemical insecticides have been recently recognized as harmful substances that cause environmental pollution and bioaccumulation, and their use has decreased [74]. Due to these environmental risks, demand for alternative control agents or biological controls with low or no environmental risks has been increasing [75, 76]. An example is bioactive substances derived from plants or natural products that have nematicidal activity against PWN. Plant essential oils or plant extracts are potential sources of bioactive chemicals as natural products for PWN control because these have few harmful effects on non-target organisms and the environment [77–82].

Since some of the above control strategies may cause problems in the ecosystem, environmentally friendly control methods have replaced the use of chemical agents. Biological control intends to control or manage PWD by using natural organisms such as predators, parasites, entomopathogenic microorganisms and fungi, and entomophilic nematodes. These can be aimed at the PWN, insect vectors, or their ectosymbiotic bacteria [83, 84]. For predators, the following insects were confirmed to feed on larvae of beetles. Predators such as *Alaus berus* (Coleoptera: Elateridae) and *Anisolabis maritima* (Dermaptera: Anisolabidiae) prey on *M. alternatus* larvae in pupal chambers, while *Temnochila japonica* (Coleopetra: Trogossitidae), *Inocellia japonica* (Neoroptera: Inoceliidae), and *Thanasinus lewisi* (Coleoptera: cleridae) are predators of *M. alternatus* larvae under the bark [85]. Several natural parasites have been found in *M. alternatus*. They are insect parasitoids such as *Scleroderma sp.* [86, 87] and *Dastarcus helophoroides* [88–91] and parasitic fungi including *Beauveria bassiana* [92–94], *B. brongniartii* and *Metarhizium anisopliae* [95–97]. Whereas *Esteya vermicola* (Ophiostomataceae) is the reported endoprarasitic fungus of the PWN, *B. xylophilus* [98–101]. *Steinernema sp.*, a parasitic nematode, is used for biological control of vector beetles [102]. However, bio-control strategies require a long time to control the beetles and the PWN. Thus, it will be necessary to use a combination of biological and chemical controls in order to achieve the desired objective of rapid control.

Conclusion

PWD by infection of the pine wood nematode *B. xylophilus* is a great threat to forest ecosystems and industries worldwide. *B. xylophilus* is a casual agent of PWD in East Asia and Europe, causing severe economic and ecological damage through deforestation and increases in pest control and forest management costs to pine forests in the world. To prevent the spread of PWD, it is crucial to identify the mechanism, such as the transmission ecology or developmental life cycle. This can help not only to understand PWD, but also to provide useful information for strategic and tactical management. In addition, it is possible to quickly prevent the spread of disease through a quick and accurate diagnosis of PWD. The diagnosis has changed from a morphological method to a method based on molecular biology, and further, the research to properly using these two methods has been conducted for rapid direct detection. Meanwhile, it is essential to build proper PWD control schemes in East Asia and Europe, as well as worldwide. Physical and chemical methods to control PWD show a high ability to control and cause mortality in the vector beetles or PWN, but these have a negative impact on the environment. Thus, biological control methods are becoming important, and research on them should be actively conducted. We now know that PWD is fatal disease in pine forests and that it can induce disastrous damage. Therefore, if we want to maintain healthy pine forests worldwide, it necessary to take further interest in and conduct research on PWD.

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Compliance with ethical standards

Conflict of interest Bit-Na Kim, Ji Hun Kim, Ji-Young Ahn, Sunchang Kim, Byung-Kwan Cho, Yang-Hoon Kim, and Jiho Min declares that they have no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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